DNA CAPILLARY ELECTROPHORESIS SETUP FOR YFILER SAMPLES USING THE EPMOTION 5075

A. SCOPE

A.1 The capillary electrophoresis setup process consists of multiple transfers of liquids containing either reagents or DNA from one place to another. By utilizing the epMotion 5075, a liquid handling robot, the incidence of human error and/or the introduction of contamination in this process can be minimized. Furthermore, automation of the capillary electrophoresis setup process allows for analysts to complete other tasks while these steps are being performed.

B. QUALITY CONTROL

B.1 A lab coat and protective gloves must be worn when performing this procedure to prevent contamination.

B.2 See DOC ID 1835 to determine reagent expiration dates.

B.3 Hi-Di formamide: To prevent repeated thawing and re-freezing of formamide, aliquot formamide into approximately 500 and 1000 µL volumes after initial thawing of the 25 mL bottle. Appropriately discard any unused aliquot of thawed formamide.

C. SAFETY

C.1 Hi-Di formamide: exposure causes eye, skin, and respiratory tract irritation. It is also a possible developmental and birth defect hazard

C.2 All appropriate SDS sheets must be read prior to performing this procedure.

C.3 Protective gloves and a lab coat must be worn when performing this procedure. Eye protection (e.g. safety glasses or a face shield) must also be worn when performing any parts of this procedure outside of the epMotion hood.

D. REAGENTS, STANDARDS AND CONTROLS

D.1 Yfiler allelic ladder

D.2 GS-500 LIZ internal size standard

D.3 Hi-Di formamide

D.4 3130 Performance Optimized Polymer (POP-4 polymer)

D.5 AB 3130 Genetic Analyzer 10X Buffer w/ EDTA.

D.5.1 To make a 1X working buffer:

Add 25 mL of 10X Buffer to 225 mL of DI H_2O to make 250 mL of working buffer

or add 100 mL of 10X Buffer (4 bottles) to 900 mL DI H_2O to make 1000 mL of working buffer.

D.6 70% ethanol (decontamination of the epMotion 5075)

D.7 DNA-ExitusPlus (decontamination of the epMotion 5075)

E. EQUIPMENT & SUPPLIES

E.1 Equipment

E.1.1 epMotion 5075 (instrument, computer, and appropriate software)

E.1.2 epMotion dispensing tools

E.1.3 epMotion labware (thermoblocks, reservoir rack, and module racks)

E.1.4 AB 3130 Genetic Analyzer (instrument, computer, and appropriate software)

E.1.5 AB 36 cm capillary array

E.1.6 AB 3130 Genetic Analyzer sample septa and plates

E.1.7 Thermal cycler

E.1.8 Pipettes

E.1.9 Vortexer

E.1.10 Frozen plate block

E.1.11 Decapper

E.1.12 96-well plate retainer and base

E.1.13 96-well plate centrifuge

E.2 Supplies

E.2.1 3130 Genetic Analyzer buffer vials/reservoirs/reservoir septa

E.2.2 Pipette tips

E.2.3 epMotion supplies (5 mL tubes, epT.I.P.S. Motion 1-50 µL tips, epT.I.P.S. Motion 20-300 µL tips)

E.2.4 Strip caps

E.2.5 Parafilm

E.2.6 50 mL conical tubes

E.2.7 Scalpel

E.2.8 Permanent marker

F. PROCEDURES

F.1 Create a 3130 plate map using the following highlighted locations for GS-500 LIZ/formamide blanks and allelic ladders.

***Note**: The 3130 plate map needs to created in such a way that air will not be injected onto the array, e.g. if 11 amplified samples are to be pipetted,* GS-500 LIZ*/formamide should still be dispensed into well D3 for the fourth capillary of the array.*

	1	2	3	4	5	6	7	8	9	10	11	12
A	*LIZ500*	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33	Sample 41	Sample 49	Sample 57	Sample 65	Sample 73	Sample 81
B	*LIZ500*	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34	Sample 42	Sample 50	Sample 58	Sample 66	Sample 74	Sample 82
C	*LIZ500*	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35	Sample 43	Sample 51	Sample 59	Sample 67	Sample 75	Sample 83
D	*LIZ500*	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36	Sample 44	Sample 52	Sample 60	Sample 68	Sample 76	Sample 84
E	*YLADDER*	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37	Sample 45	Sample 53	Sample 61	Sample 69	Sample 77	Sample 85
F	*YLADDER*	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38	Sample 46	Sample 54	Sample 62	Sample 70	Sample 78	Sample 86
G	*YLADDER*	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39	Sample 47	Sample 55	Sample 63	Sample 71	Sample 79	Sample 87
H	*YLADDER*	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40	Sample 48	Sample 56	Sample 64	Sample 72	Sample 80	Sample 88

F.2 Import or create a plate document as described in sections F.3 and F.4 of DOC ID 1766. The plate document does not have to be prepared at this step; it can be prepared at any time.

F.3 Combine the necessary amount of formamide and GS-500 LIZ in an Eppendorf 5 mL tube as follows:

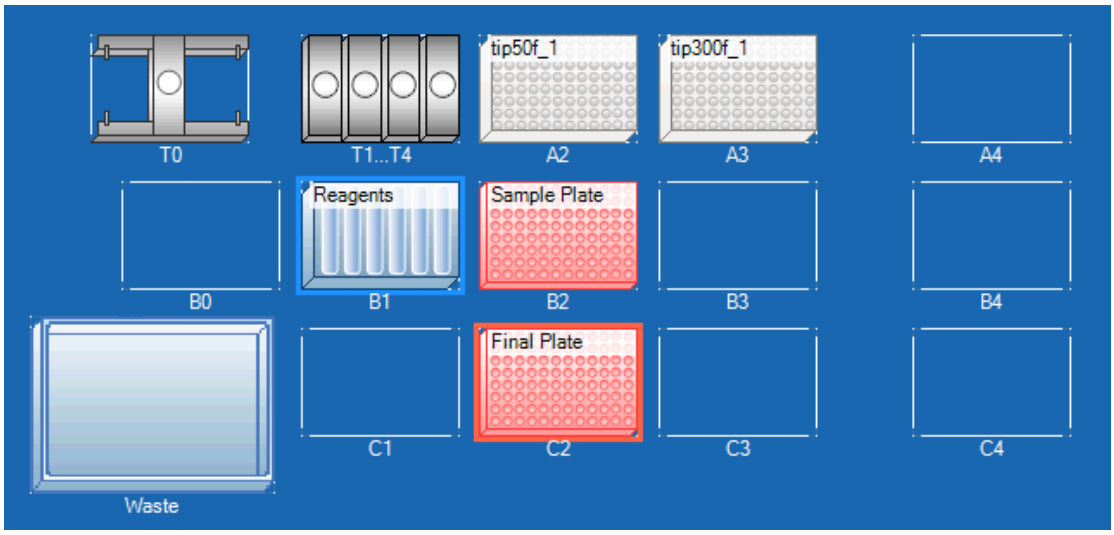
(Number of samples + 2) x 24.5 µL formamide

(Number of samples + 2) x 0.5 µL GS-500 LIZ

***Note**: It is recommended that enough volume for additional samples be included in the calculation to account for volume lost in pipetting.*

F.4 Briefly vortex the mixture. Prepare the epMotion worktable with tips, dispensing tools, the GlobalFiler amplification plate (with strip caps removed) and an empty 96-well plate as shown below; Note: The amplification plates must be centrifuged briefly before placing them on the robot worktable; these plates may also be added to the worktable after preparing the reagent reservoir rack, i.e. after Step F.5.2.

***Note**: A minimum volume of 8 µL of amplified product is required for this epMotion protocol.*



F.5 Prepare the reagent reservoir rack:

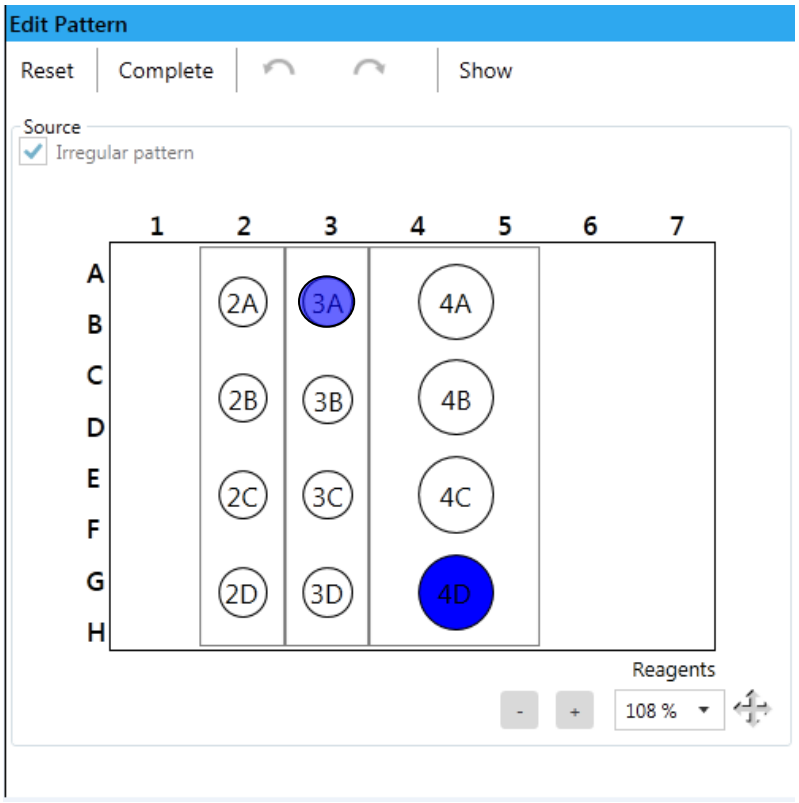
F.5.1 Ensure that a 1.5/2 mL module rack is located in both the second and the third positions of the reservoir rack as shown below.

F.5.2 Ensure that a 5 mL module rack is present in the fourth and fifth positions (this larger rack takes up two positions).

F.5.3 Place the opened 5 mL tube containing the GS-500 LIZ /formamide master mix in position 4D of the reservoir rack with the tube lid in the lid holder.

F.5.4 Place an opened tube containing Yfiler allelic ladder in position 3A of the reservoir rack.

***Note**: A minimum volume of 13 µL of allelic ladder is required for this epMotion protocol.*



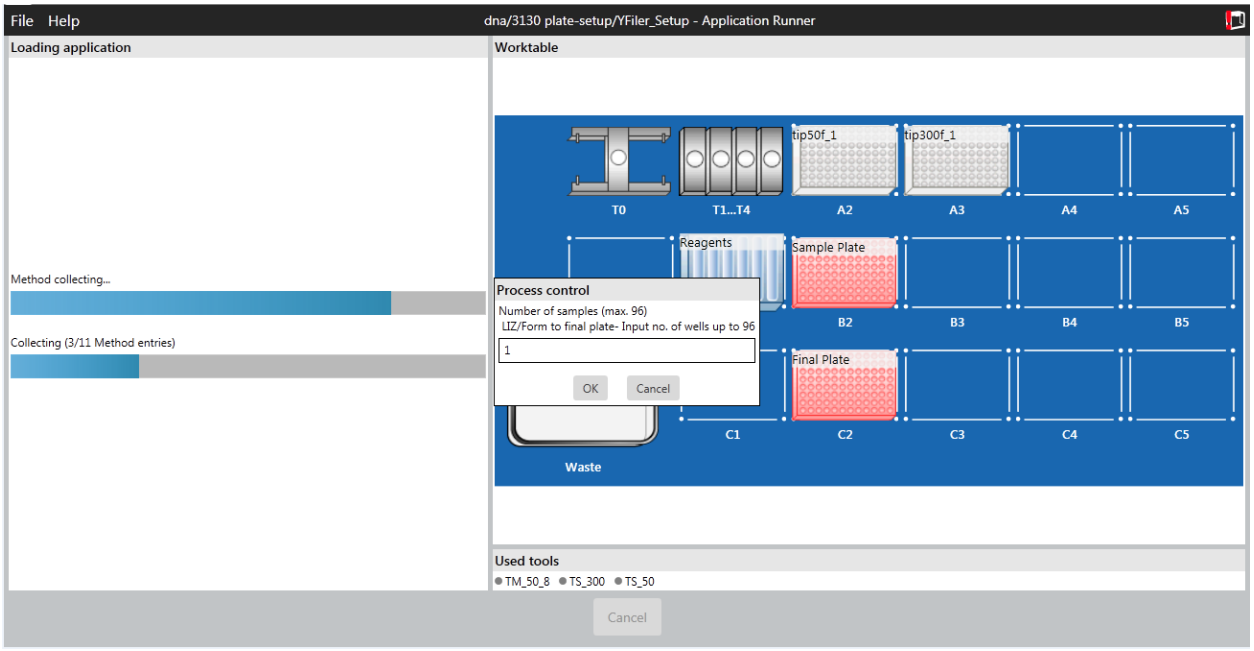
F.6 Close the front hood of the epMotion.

F.7 Open the Eppendorf eBlue software.

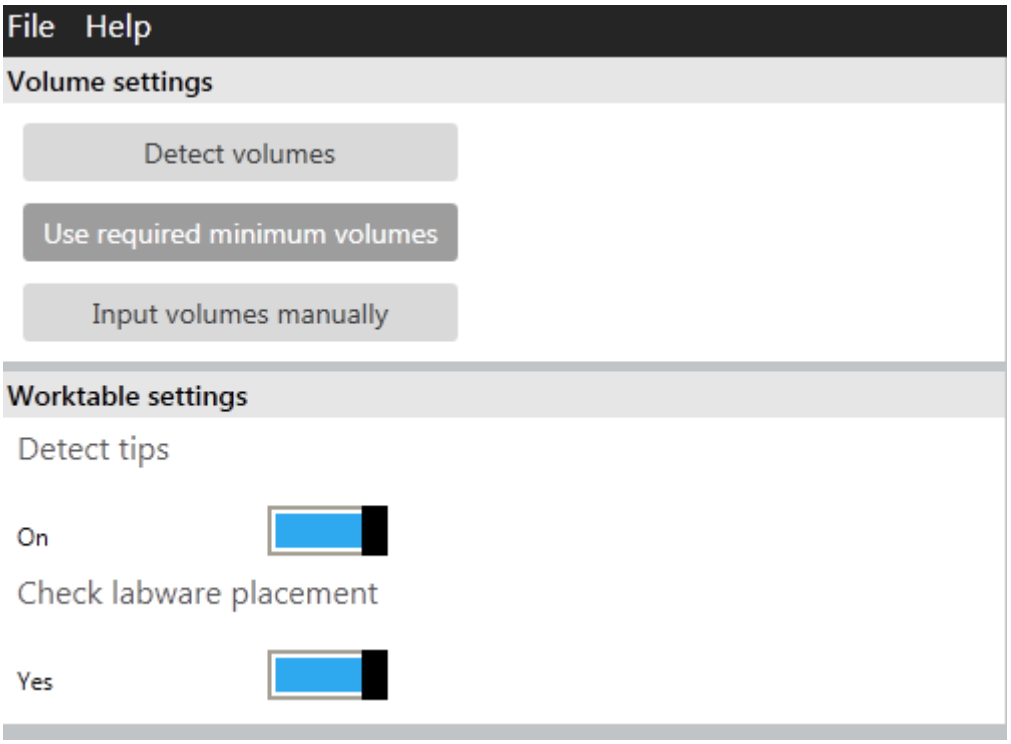
F.8 Select **Application Runner** from the main menu, choose the YFiler_Setup.dws method in the 3130 folder of the DNA account.

F.9 Ensure that compatible devices is selected and that device 5075ZN301615 is highlighted, click **Next**.

F.10 The method will start and you will be prompted twice; first to input the number of wells the LIZ500/formamide will be added to the final plate (up to 96), second to input the number of PCR products to add to the final plate (including any blanks to fill columns, up to 88) example of the first prompt below:



F.11 Ensure that Use required minimum volumes, Detect tips, and Check labware placement are selected on the following screen, click run.



F.12 To stop the method before it is complete, click the **Pause Icon** in the Control tab or lift the front hood. Then click the **Abort icon** to abort the method; the front hood must be down to abort the method. Alternatively, after stopping the method, click the **Continue Icon** to continue the method.

F.13 After completion of the method, click **OK**, click on **Log Viewer** select your log file by finding the file with the appropriate date and time. Select the **Print Icon** and click **PDF** to save the file as a PDF document. This file should be saved in the appropriate analyst's casework folder on the I drive. A run completed without errors will have "Program ended successfully" on the last line of the log file.

F.14 Open the front hood and remove the 3130 plate and place it into a plate base. Cover the plate with a rubber septa.

F.15 Centrifuge the plate briefly.

F.16 Heat the samples in a thermal cycler for three minutes at 95°C to denature.

F.17 Snap-cool immediately for a minimum of three minutes in a frozen plate holder.

F.18 Place the plate into the plate base and centrifuge briefly.

F.19 Secure the 3130 plate with a plastic retainer clip.

F.20 Place the tray onto the 3130 autosampler with position A1 at the back right.

F.21 Link the 3130 plate to a run by clicking on the yellow plate diagram. Select the green arrow from the Run Scheduler window to start the run; see sections F.5 of DOC ID 1766 for additional information.

Note: When the run is complete, a copy of the raw data should be saved in the appropriate analyst's casework folder on the I drive. The analyst should maintain case folders in monthly files. The data from the first analysis of any convicted offender or arrestee sample should be stored under K:\Division\DNA\CODIS\Analysis.

F.22 Prepare the epMotion worktable for the next user:

F.22.1 Cap the allelic ladder and remove it from the reservoir rack.

F.22.2 Discard any unused GS-500 LIZ/formamide mixture.

F.22.3 Empty the waste container.

F.22.4 Wipe down the epMotion deck with 70% ethanol solution or DNA Exitus Plus cleaning solution.

G. INTERPRETATION GUIDELINES

G.1 See DOC ID 1776 (Yfiler Interpretation Guidelines).

H. REFERENCES

H.1 Eppendorf epMotion 5075 with Integrated PC and epBlue Operating Manual, 2008.

H.2 epMotion Validation Binder 3, YFiler_Setup.dws method.